Biochemical and Biophysical Research Communications xxx (2016) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

In vitro reconstitution of breast cancer heterogeneity with multipotent cancer stem cells using small molecules

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ARTICLE INFO

Article history:
Received 10 November 2016
Accepted 17 November 2016
Available online xxx

Keywords:
Mammary tumor
Heterogeneity
Multipotency
Small molecules
Metaplasia

ABSTRACT

A small fraction of tumor cells are thought to possess the potential for both multiple-lineage differentiation and self-renewal, which underlies the cancer stem cell hypothesis. However, the differentiation mechanisms of these cells have not been elucidated due to a lack of appropriate culture methods. Here, we established a culture condition for maintaining multipotent tumor cells from rat breast tumors using 4 small molecules. Cultured tumor cells in this condition retained their intrinsic myoepithelial features, expressing p63 and CK14 and vimentin. In a xenograft model, the p63-expressing cells formed epithelial tumors containing glandular, squamous and sebaceous compartments. Upon withdrawal of the small molecules, p63 and CK14 expression was lost, with concurrent increase in expression of mesenchymal markers. These transited cells acquired drug resistance and invasiveness and showed massive sarcomatoid tumorigenicity. Epithelial features could not be recovered by re-exposure to the small molecules in the transited cells. Here, we have identified multipotent cancer cells within primary mammary tumors and demonstrated that their plasticity is maintained by the small molecules.

1. Introduction

Cancer tissue consists of heterogeneous cancer cells with diverse characteristics. Recent studies have proposed the concept of cancer stem cells (CSCs) to mainly explain the heterogeneity regarding tumorigenic and nontumorigenic cancer cells [1]. Meanwhile, CSCs are defined as cells within a tumor that possess the capacity to undergo self-renewal and to give rise to the heterogeneous lineages of cancer cells that comprise the tumor. Histological and genetic analyses of these cancer tissues reveal that a subset of solid cancers has the potential to differentiate into numerous cell lineages, as represented by metaplasia, suggesting that CSCs may have multi-lineage differentiation properties [2–4]. However, the multipotent potential of CSCs and the mechanisms underlying the control of CSC differentiation in solid cancers remain largely unclear. Although both small molecules and growth factors are reported to be useful in inducing or blocking the differentiation of tumor initiating cells [5–7], the difficulty in uncovering the mechanisms of CSC differentiation can be attributed to a lack of culture methods for expanding CSCs while maintaining their multipotency. Identifying methods for culturing multipotent cancer cells is biologically important to understand the maintenance of cancer cell stemness and the cell fate transitions of cancer cells, which may eventually contribute to the development of CSC-targeting cancer therapeutics.

Metaplastic carcinoma of the breast is a variant of breast cancer in which the components of the neoplasm have an appearance other than that of glandular epithelium, resulting in a heterogeneous cancer tissue with multiple cell lineages. Given the monoclonal origin of cancer [2,3], multi-lineage differentiation in a single tumor indicates the presence of multipotent CSCs in breast cancers. Previous works reported that human mammary epithelial cells that were transformed by oncogenes showed the potential for squamous cell differentiation in xenograft models [8,9]. Similarly, Keller et al. reported that transformation of myoepithelial cells resulted in the development of metaplastic tumors associated with squamous, sebaceous and spindle cell differentiation. These results raised the possibility that myoepithelial cells can adopt multipotent CSC states to form metaplastic carcinoma-like tumors [10,11]. However, it is not clear whether such multipotent cancer cells exist in primary mammary tumors and if multipotent cancer cell lines can be established from the primary tumors *in vitro*.

Our previous work made it possible to establish rat ES cells

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http://dx.doi.org/10.1016/j.bbrc.2016.11.106
0006-291X/

using a combination of four small molecules, Y-27632, PD0325901, A-83-01 and CHIR99021 (termed YPAC), which inhibit ROCK, MEK, Tgf-β and GSK3, respectively [12]. Our findings that YPAC strongly inhibits differentiation and enhances self-renewal of rat pluripotent stem cells suggest that YPAC might exert similar effects on other types of stem cells, including multipotent CSCs. To examine the multipotent nature of cancer cells, in the present study, we cultured rat mammary tumors from the $p53^{+/-}$ background by utilizing YPAC.

2. Material and methods

2.1. Media, animals, and primers

The YPAC medium was prepared by the addition of 10 μM Y-27632 (WAKO), 1 μM PD0325901 (Axon Medchem), 0.5 μM A-83-01 (TOCRIS), and 3 μM CHIR99021 (Axon Medchem) to basic culture medium. The basic medium was composed of DMEM (including 110 mg/L sodium pyruvate and 200 mM L-glutamine, GIBCO), 20% FBS (Lot No. 871470, GIBCO) or 20% KSR (GIBCO), 0.1 mM 2-mercaptoethanol (Sigma-Aldrich), 1% nonessential amino acid stock (GIBCO), and 1× antibiotic antimycotic (GIBCO). The -i cells of rat mammary tumor were established using a medium composed of DMEM, 10% FBS and 1× antibiotic antimycotic. Animal experiments were performed in compliance with the guidelines of the Institute for Laboratory Animal Research, National Cancer Center Research Institute. These studies were approved by the National Cancer Center Research Institute. All primer sequences are listed in Supplementary Table S1.

2.2. Primary culture of rat mammary tumor

Tumor cells were isolated from rat mammary tumors caused in an 11-month-old $p53^{+/-}$ female rat. The tumor was cut into 1-mm^3 pieces with scissors, and the minced tissues were seeded on collagen I-coated dished under various culture conditions. Two

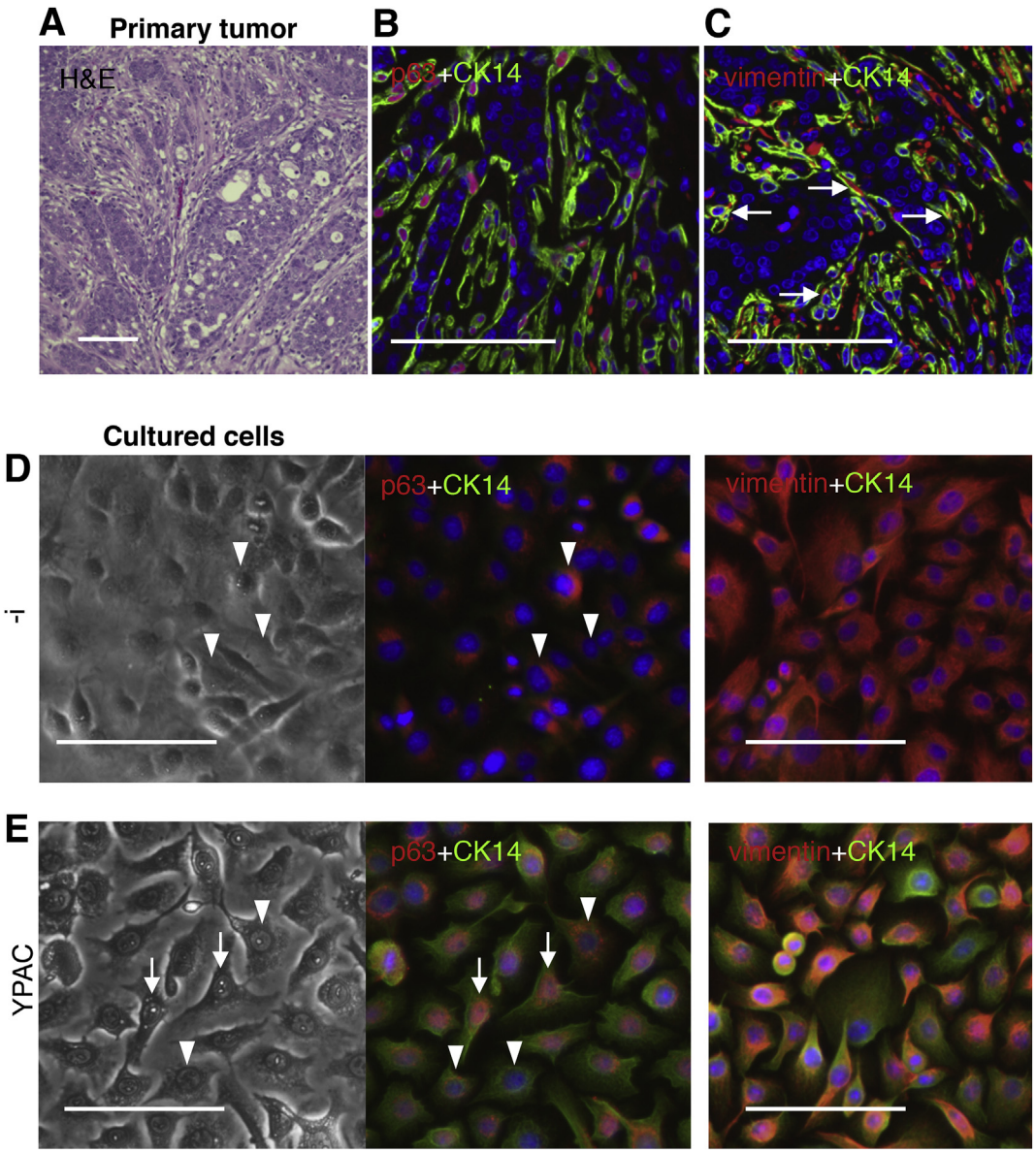

Fig. 1. Histology of mammary tumor and cultured tumor cells. (A) H&E staining of a mammary tumor developed in a $p53^{+/-}$ rat. (B, C) IHC of mammary tumor sections for (B) p63 (red) and CK14 (green) or (C) vimentin (red) and CK14 (green). Characterization of mammary tumor cells cultured under (D) -i and (E) YPAC conditions. ICC for p63 (red) and CK14 (green) or (C) vimentin (red) and CK14 (green) was performed. Arrows indicate cells showing nuclear p63. Arrowheads indicate nuclear p63 negative cells. All scale bars = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Please cite this article in press as: M. Kawamata, et al., *In vitro* reconstitution of breast cancer heterogeneity with multipotent cancer stem cells using small molecules, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.11.106